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Structure activity relationships of human galactokinase inhibitors

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ABSTRACT

Classic Galactosemia is a rare inborn error of metabolism that is caused by deficiency of galactose-1-phosphate uridyltransferase (GALT), an enzyme within the Leloir pathway that is responsible for the conversion of galactose-1-phosphate (gal-1-p) and UDP-glucose to glucose-1-phosphate and UDP-galactose. This deficiency results in elevated intracellular concentrations of its substrate, gal-1-p, and this increased concentration is believed to be the major pathogenic mechanism in Classic Galactosemia. Galactokinase (GALK) is an upstream enzyme of GALT in the Leloir pathway and is responsible for conversion of galactose and ATP to gal-1-p and ADP. Therefore, it was hypothesized that the identification of a small-molecule inhibitor of human GALK would act to prevent the accumulation of gal-1-p and offer a novel entry therapy for this disorder. Herein we describe a quantitative high-throughput screening campaign that identified a single chemotype that was optimized and validated as a GALK inhibitor.

The galactosemias are rare inherited metabolic disorders caused by deficiencies of the enzymes in the Leloir Pathway.[1,2] As the Leloir Pathway is the predominate mechanism for the metabolism of galactose, all of the galactosemias result in aberrant levels of galactose and/or its down-stream metabolites (Fig. 1). Classic Galactosemia (Type I) is the most common form of the galactosemias and is also the most severe. It is a potentially lethal disorder with a high mortality rate when left untreated. Currently, many state newborn screening programs include testing for galactosemia, and if detected, immediate removal of lactose and galactose from an infant's diet is required.[3] With widespread incorporation of this screening, the morbidity rates for those affected with Classic Galactosemia has significantly decreased, but the life-long galactose restricted diet fails to prevent developmental delay, neurological disorders, and premature ovarian insufficiency, which occur in many affected patients later in life.[4–7] Classic Galactosemia is characterized by deficient galactose-1-phosphate uridyltransferase (GALT), which results in the buildup of its substrate galactose-1-phosphate (gal-1-P).[8] Although the exact pathogenic mechanism of Classic Galactosemia has not been established, elevated galactose-1-phosphate (gal-1-p) levels have been proposed as a major pathogenic factor.[3,9–11] Immediately upstream from GALT is galactokinase (GALK), which is the enzyme responsible for converting galactose into gal-1-p. Deficiency in the GALK enzyme results in Type II Galactosemia and these GALK-deficient patients have much milder and even benign phenotypes.[12] This positions GALK as a target for reduction of gal-1-p levels and a potential therapeutic target for Classic Galactosemia.[13,14]

Using recombinant GALK1 we performed a quantitative high-throughput screen (qHTS) against ~274,000 compounds in 1536-well plate format at 6 doses (3 nM–57 μM).[13,15] ATP was held at 35 μM and galactose at 100 μM, both near their K_m values determined under the 1536-well assay conditions. We used the KinaseGlo™ detection system, which measures remaining ATP levels after conversion of galactose to gal-1p by GALK1. While the screen performed acceptably ($Z' = 0.48 \pm 0.16$, signal/background = 4.5 ± 1.6 and CV = 17 ± 9), there was an extremely low initial hit rate of 0.05%. Counter-screens were run to identify potential false positives with activity against the KinaseGlo detection reagent itself in ATP containing buffer; and with interfering redox activity, as measured by H_2O_2 production in an HRP-phenol red coupled assay (PubChem AIDs 1379 and 2502, respectively). With the exclusion of these two classes of potential artifacts only 65

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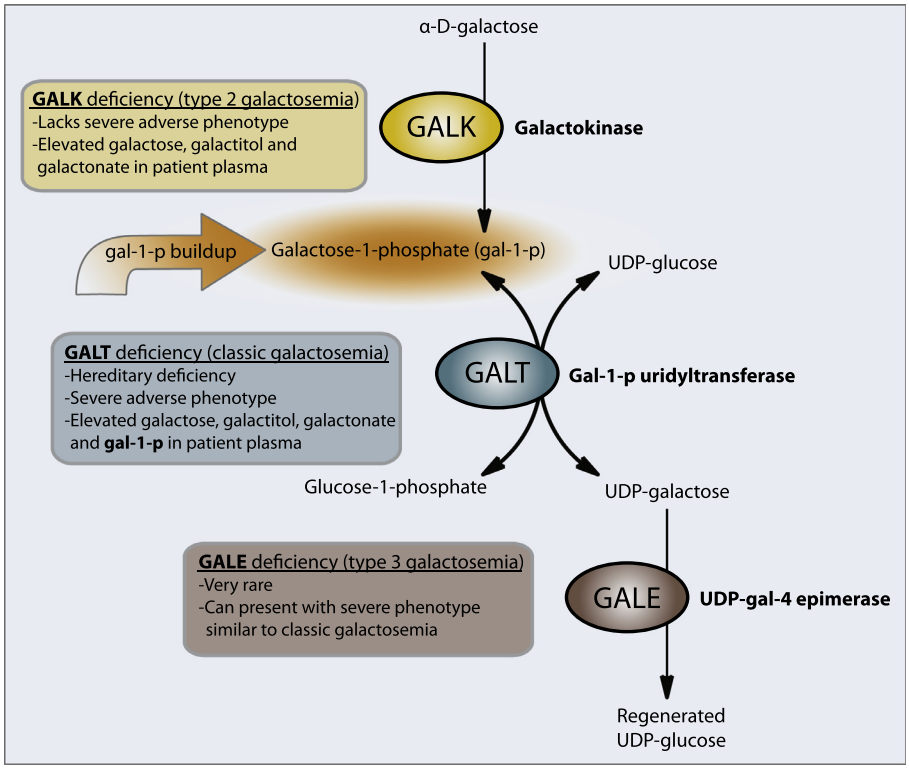
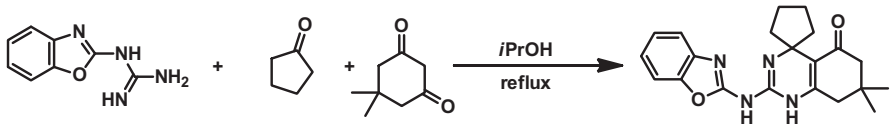


Figure 1. The Leloir pathway for galactose metabolism, highlighting the deficient enzymes responsible for the galactosemias.

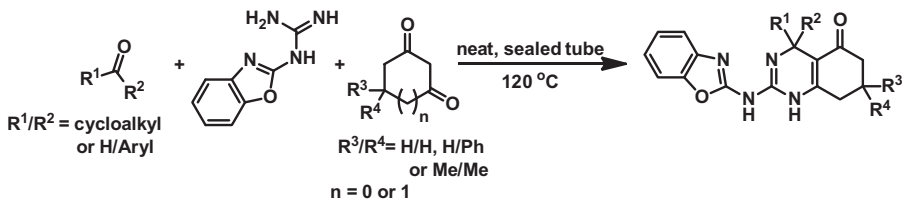
compounds remained. Within this small list of compounds, the spiro-benzoxazole series, exemplified by **1** (PubChem CID 1286615), emerged as a validated hit series. Upon resynthesis of **1** using the one-pot procedure reported by Potapov et al. (Scheme 1),[16] we confirmed the GALK1 inhibitory activity with an IC_{50} of 6 μM and began initial structure activity relationship (SAR) exploration.

While the resynthesis of **1** performed well in refluxing isopropanol, the chemistry did not translate well to more diverse substrates. After unsuccessfully trying a few acid and base catalysts, we settled on mixing the three components neat, sealing in a microwave vial, and submerging into a 120 °C oil bath (Scheme 2). This protocol allowed for rapid access to a number of analogs for initial SAR (Table 1).

We first looked at the importance of the gem-dimethyl group and found that replacement with a mono-phenyl substituent (**2**) gave reduced activity, while complete removal of the methyl groups gave improved activity to 1 μM (**3**). Contracting the fused cyclohexenone ring to a 5-membered ring (**4**) reduced activity. Next, we looked at the spiro ring and made cyclohexane (**5**), dimethyl (**6**) and cyclobutane (**7**) analogs. While all three analogs maintained GALK1 inhibitory activity, they were significantly weaker than **3**. Attempts to replace the benzoxazole were limited by the chemistry in this multi-component reaction, but we were able to access two guanidinyl benzimidazoles (**8** and **9**) through chemistry outlined in Scheme 3, followed by the standard neat melting chemistry typified in Scheme 2. Unfortunately, both analogs were inactive. Due to the low solubility of **3** and with



Scheme 1. Resynthesis of **1**.



Scheme 2. Use of neat melting procedure for broader applicability to diverse substrates.